A 32

The sewed product was digested BglII/NotI and ligated into the BamHI/NotI sites of pGEX6P-1. –

Please replace the paragraph beginning at page 73, line 19, with the following rewritten paragraph:

A 33

– All components used for purification of the GFP gene products were from Pharmacia Biotec (Piscataway, NJ) except as noted. The human codon-optimized gene for each protein was expressed in BL21 TIL codon plus (DE3) *E. coli* (Stratagene, San Diego, CA) as a fusion protein with glutathione S-transferase from pGEX6p-1 derived vectors. Each protein was purified using Glutathione Sepharose 4B beads as per the manufacturer's directions, and the mature GFP was removed from the protein with Precision Protease. The purified proteins ran as single bands by SDS-PAGE and appeared as single peaks of the expected molecular mass by MALDI-TOF mass spectometry on a Bruker Reflex III instrument (Bruker Daltonics, Billerica, MA). Due to the cloning strategy, purified *R. muelleri* GFP has the amino acids PLGSEF- (SEQ ID NO:84) and *Ptilosarcus* GFP the residues GPLGS- (SEQ ID NO:85) fused to their N-termini. Purified recombinant EGFP was from Clontech (Palo Alto, CA). –

REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

These amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disc containing the above named sequence, SEQUENCE ID NUMBERS 1-85 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "Patent-In" provided by the PTO. The information contained in the computer readable disc

is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

The Commissioner is authorized to charge any fees, including extension fees, which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-68531-1/DJB/RMS/AMS).

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: April 10, 2001

Robin M. Silva
Reg. 38,304

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

S rial No.: 09/710,058
Filed: November 10, 2000

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph beginning at page 2, line 18, has been amended as follows:

– Figure 1 (SEQ ID NOS:2, 5-8) depicts a homology lineup between the GFPs of *Renilla Mulleri* (SEQ ID NO:2), *Pitilosarcus Gurneyi* (SEQ ID NO:5), *Aequorea* (SEQ ID NO:7) and its enhanced version, EGFP (SEQ ID NO:6). The underlined residues are the fluorescent tripeptide (chromophore). Identity, strong similarity and weak similarity are depicted. –

Paragraph beginning at page 2, line 21, has been amended as follows:

– Figure 2 depicts the nucleic acid sequence (SEQ ID NO:1) of the wild type rGFP (SEQ ID NO:2). –

Paragraph beginning at page 2, line 22, has been amended as follows:

– Figure 3 depicts the nucleic acid sequence (SEQ ID NOS:3, 4) of the wild type pGFP (SEQ ID NO:5). –

Paragraph beginning at page 2, line 24, has been amended as follows:

– The present invention is directed to the use of Renilla green fluorescent protein (hereinafter "rGFP"), in a variety of methods and compositions that exploit the

autofluorescent properties of rGFP. These methods include, but are not limited to, the use of rGFP as a reporter molecule in cell screening assays, including intracellular assays; the use of rGFP as a scaffold protein for fusions with random peptide libraries; etc. Similarly, compositions of rGFP are provided, including constructs of rGFP such as fusion constructs that include rGFP as a reporter gene, retroviral constructs including rGFP and internal ribosome entry sites (IRES), etc. Basically, the invention provides a number of novel uses for rGFP, similar to those outlined for aGFP in WO 95/07463, hereby incorporated by reference in its entirety. In addition, the invention is also directed to the use of Pitilosarcus Gurneyi green fluorescent protein ("pGFP"), the amino acid sequence of which is shown in Figure 1 (SEQ ID NO:5) and is also depicted in WO 99/49019. It should be noted that while the discussion below is directed to rGFP, pGFP may be used as well. –

Paragraph beginning at page 3, line 10, has been amended as follows:

– In a preferred embodiment, the invention provides compositions including rGFP. By "Renilla green fluorescent protein" or "rGFP" herein is meant a protein that has significant homology, as defined herein, to the wild-type protein of Figure 1 (SEQ ID NO:2), as depicted in WO 99/49019, hereby incorporated by reference in its entirety. –

Paragraph beginning at page 3, line 14, has been amended as follows:

– In a preferred embodiment, the invention provides compositions including pGFP. By Ptilosarcus green fluorescent protein" or "pGFP" herein is meant a protein that has significant homology, as defined herein, to the wild-type protein of Figure 3 (SEQ ID NO:5), as depicted in WO 99/49019, hereby incorporated by reference in its entirety. –

Paragraph beginning at page 3, line 18, has been amended as follows:

– An rGFP or pGFP protein of the present invention may be identified in several ways. "Protein" in this sense includes proteins, polypeptides, and peptides. A r- or pGFP nucleic acid or protein is initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in Figures 1, 2 and 3 (SEQ ID NOS:1-8). Such homology can be based upon the overall nucleic acid or amino acid sequence. –

Paragraph beginning at page 3, line 23, has been amended as follows:

– As used herein, a protein is a "rGFP protein" or "pGFP" if the overall homology of the protein sequence to the amino acid sequence shown in Figures 2 or 3 (SEQ ID NOS:2, 5) is preferably greater than about 75%, more preferably greater than about 80%, even more preferably greater than about 85% and most preferably greater than 90%. In some embodiments the homology will be as high as about 93 to 95 or 98%. –

Paragraph beginning at page 5, line 5, has been amended as follows:

– The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the protein sequences shown in Figure 1 (SEQ ID NOS:2, 5-8), it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in Figure 1 (SEQ ID NOS:2, 5-8), as discussed below, will be determined using the number of amino acids in the shorter sequence. –

Paragraph beginning at page 5, line 11, has been amended as follows:

– GFP proteins of the present invention may be shorter or longer than the amino acid sequences shown in Figure 1 (SEQ ID NOS:2, 5-8). Thus, in a preferred embodiment, included within the definition of GFP proteins are portions or fragments of the sequences depicted herein. Portions or fragments of r- and pGFP proteins are considered GFP proteins if a) they share at least one antigenic epitope; or b) have at least the indicated homology; c) preferably have GFP biological activity, e.g., including, but not limited to, autofluorescence; or d) fold into a stable structure that is similar to the wild-type structure.–

Paragraph beginning at page 13, line 5, has been amended as follows:

– A preferred coiled-coil presentation structure is as follows:

MGCAALESEVSALESEVASLESEVAAL**GRGDMP**LAAVKSKLSAVKSKLASVKSKLAACG PP (SEQ ID NO:9). The underlined regions represent a coiled-coil leucine zipper region defined previously (see Martin et al., EMBO J. 13(22):5303-5309 (1994), incorporated by reference). The bolded GRGDMP (SEQ ID NO:10) region represents the loop structure and when appropriately replaced with randomized peptides (i.e. peptides, generally depicted herein as $(X)_n$, where X is an amino acid residue and n is an integer of at least 5 or 6) can be of variable length. The replacement of the bolded region is facilitated by encoding restriction endonuclease sites in the underlined regions, which allows the direct incorporation of randomized oligonucleotides at these positions. For example, a preferred

embodiment generates a XhoI site at the double underlined LE site and a HindIII site at the double-underlined KL site. –

Paragraph beginning at page13, line 23, has been amended as follows:

– A preferred minibody presentation structure is as follows:

MGRNSQATS**GFT***F***SHF**YMEWVRGGEYIAASR**HKHNKY**TTEYSASVKGRYIVSRDTSQS ILYLQKKKGPP (SEQ ID NO:11). The bold, underline regions are the regions which may be randomized. The ~~italized~~ italicized phenylalanine must be invariant in the first randomizing region. The entire peptide is cloned in a three-oligonucleotide variation of the coiled-coil embodiment, thus allowing two different randomizing regions to be incorporated simultaneously. This embodiment utilizes non-palindromic BstXI sites on the termini.–

Paragraph beginning at page14, line 6, has been amended as follows:

– In a preferred embodiment, the presentation sequence confers the ability to bind metal ions to confer secondary structure. Thus, for example, C2H2 zinc finger sequences are used; C2H2 sequences have two cysteines and two histidines placed such that a zinc ion is chelated. Zinc finger domains are known to occur independently in multiple zinc-finger peptides to form structurally independent, flexibly linked domains. See J. Mol. Biol. 228:619 (1992). A general consensus sequence is (5 amino acids)-C-(2 to 3 amino acids)-C-(4 to 12 amino acids)-H-(3 amino acids)-H-(5 amino acids). A preferred example would be -FQCEEC-random peptide of 3 to 20 amino acids-HIRSHTG- (SEQ ID NO:12).–

Paragraph beginning at page 14, line 13, has been amended as follows:

– Similarly, CCHC boxes can be used (see Biochem. Biophys. Res. Commun. 242:385 (1998)), that have a consensus ~~seqeunce~~ sequence -C-(2 amino acids)-C-(4 to 20 random peptide)-H-(4 amino acids)-C- (see Bavoso et al., Biochem. Biophys. Res. Comm. 242(2):385 (1998), hereby incorporated by reference. Preferred examples include (1) -VKCFNC-4 to 20 random amino acids-HTARNCR- (SEQ ID NO:13), based on the nucleocapsid protein P2; (2) a sequence modified from ~~tehat~~ that of the naturally ~~occuring~~ occurring zinc-binding peptide of the Lasp-1 LIM domain (Hammarstrom et al., Biochem. 35:12723 (1996)); and (3) -MNPNCARCG-4 to 20 random amino acids-HKACF- (SEQ ID NO:14), based on the nmr structural ensemble 1ZFP (Hammarstrom et al., Biochem. 35 U.S.C. 35(39):12723 (1996). –

Paragraph beginning at page 15, line 9, has been amended as follows:

– Suitable dimerization sequences will encompass a wide variety of sequences. Any number of protein-protein interaction sites are known. In addition, dimerization sequences may also be elucidated using standard methods such as the yeast two hybrid system, traditional biochemical affinity binding studies, or even using the present methods. See U.S.S.N. 60/080,444, filed April 2, 1998, hereby incorporated by reference in its ~~entireity~~ entirety. Particularly preferred dimerization peptide sequences include, but are not limited to, -EFLIVKS- (SEQ ID NO:15), EEFLIVKKS- (SEQ ID NO:16), -FESIKLV- (SEQ ID NO:17), and -VSIKFEL- (SEQ ID NO:18). –

Paragraph beginning at page 16, line 1, has been amended as follows:

– In a preferred embodiment, the targeting sequence is a nuclear localization signal (NLS). NLSs are generally short, positively charged (basic) domains that serve to direct the entire protein in which they occur to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic NLS's such as that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val (SEQ ID NO:19)), Kalderon (1984), et al., Cell, 39:499-509; the human retinoic acid receptor-ß nuclear localization signal (ARRRRP (SEQ ID NO:20)); NFκB p50 (EEVQRKRQKL (SEQ ID NO:21); Ghosh et al., Cell 62:1019 (1990); NFκB p65 (EEKRKRTYE (SEQ ID NO:22); Nolan et al., Cell 64:961 (1991); and others (see for example Boulikas, J. Cell. Biochem. 55(1):32-58 (1994), hereby incorporated by reference) and double basic NLS's exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp (SEQ ID NO:23)), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that NLSs incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann, Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990. –

Paragraph beginning at page 17, line 12, has been amended as follows:

– Useful sequences include sequences from: 1) class I integral membrane proteins such as IL-2 receptor beta-chain (residues 1-26 are the signal sequence, 241-265 are the transmembrane residues; see Hatakeyama et al., Science 244:551 (1989) and von Heijne

et al, Eur. J. Biochem. 174:671 (1988)) and insulin receptor β-chain (residues 1-27 are the signal, 957-959 are the transmembrane domain and 960-1382 are the cytoplasmic domain; see Hatakeyama, supra, and Ebina et al., Cell 40:747 (1985)); 2) class II integral membrane proteins such as neutral endopeptidase (residues 29-51 are the transmembrane domain, 2-28 are the cytoplasmic domain; see Malfroy et al., Biochem. Biophys. Res. Commun. 144:59 (1987)); 3) type III proteins such as human cytochrome P450 NF25 (Hatakeyama, supra); and 4) type IV proteins such as human P-glycoprotein (Hatakeyama, supra). Particularly preferred are CD8 and ICAM-2. For example, the signal sequences from CD8 and ICAM-2 lie at the extreme 5' end of the transcript. These consist of the amino acids 1-32 in the case of CD8 (MASPLTRFLSLNLLLLGESILGSGEAKPQAP (SEQ ID NO:24); Nakauchi et al., PNAS USA 82:5126 (1985) and 1-21 in the case of ICAM-2 (MSSFGYRTLTVALFTLICCPG (SEQ ID NO:25); Staunton et al., Nature (London) 339:61 (1989)). These leader sequences deliver the construct to the membrane while the hydrophobic transmembrane domains, placed 3' of the random peptide region, serve to anchor the construct in the membrane. These transmembrane domains are encompassed by amino acids 145-195 from CD8 (PQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALLLSLIITLICYHSR (SEQ ID NO:26); Nakauchi, supra) and 224-256 from ICAM-2 (MVIIVTVVSVLLSLFVTSVLLCFIFGQHLRQQR (SEQ ID NO:27); Staunton, supra). –

Paragraph beginning at page 17, line 31, has been amended as follows:

– Alternatively, membrane anchoring sequences include the GPI anchor, which results in a covalent bond between the molecule and the lipid bilayer via a glycosyl-phosphatidylinositol bond for example in DAF

(PNKGSGTT**S**GTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO:28), with the bolded serine the site of the anchor; see Homans et al., Nature 333(6170):269-72 (1988), and Moran et al., J. Biol. Chem. 266:1250 (1991)). In order to do this, the GPI sequence from Thy-1 can be cassetted 3' of the variable region in place of a transmembrane sequence. –

Paragraph beginning at page 18, line 3, has been amended as follows:

– Similarly, myristylation sequences can serve as membrane anchoring sequences. It is known that the myristylation of c-src recruits it to the plasma membrane. This is a simple and effective method of membrane localization, given that the first 14 amino acids of the protein are solely responsible for this function: MGSSKSKPKDPSQR (SEQ ID NO:29) (see Cross et al., Mol. Cell. Biol. 4(9):1834 (1984); Spencer et al., Science 262:1019-1024 (1993), both of which are hereby incorporated by reference). This motif has already been shown to be effective in the localization of reporter genes and can be used to anchor the zeta chain of the TCR. This motif is placed 5' of the variable region in order to localize the construct to the plasma membrane. Other modifications such as palmitoylation can be used to anchor constructs in the plasma membrane; for example, palmitoylation sequences from the G protein-coupled receptor kinase GRK6 sequence (LLQRLFSRQD**CC**GN**C**SDSEEELPTRL (SEQ IDNO:30), with the bold cysteines being palmitolyated; Stoffel et al., J. Biol. Chem 269:27791 (1994)); from rhodopsin (KQFRNCMLTSL**CC**GKNPLGD (SEQ ID NO:31); Barnstable et al., J. Mol. Neurosci. 5(3):207 (1994)); and the p21 H-ras 1 protein (LNPPDESGPGCMS**C**KCVLS (SEQ ID NO:32); Capon et al., Nature 302:33 (1983)). –

Paragraph beginning at page 18, line 16, has been amended as follows:

– In a preferred embodiment, the targeting sequence is a lysozomal targeting sequence, including, for example, a lysosomal degradation sequence such as Lamp-2 (KFERQ (SEQ ID NO:33); Dice, Ann. N.Y. Acad. Sci. 674:58 (1992); or lysosomal membrane sequences from Lamp-1 (*MLIPIAGFFALAGLVLIVLIAYLI*GRKRSHAGYQTI (SEQ ID NO:34), Uthayakumar et al., Cell. Mol. Biol. Res. 41:405 (1995)) or Lamp-2 (*LVPIAVGAALAGVLILVLLAYFIG*LKHHHAGYEQF (SEQ ID NO:35), Konecki et la., Biochem. Biophys. Res. Comm. 205:1-5 (1994), both of which show the transmembrane domains in italics and the cytoplasmic targeting signal underlined). –

Paragraph beginning at page 18, line 23, has been amended as follows:

– Alternatively, the targeting sequence may be a ~~mitrochondrial~~ mitochondrial localization sequence, including mitochondrial matrix sequences (e.g. yeast alcohol dehydrogenase III; MLRTSSLFTRRVQPSLFSRNILRLQST (SEQ ID NO:36); Schatz, Eur. J. Biochem. 165:1-6 (1987)); mitochondrial inner membrane sequences (yeast cytochrome c oxidase subunit IV; MLSLRQSIRFFKPATRTLCSSRYLL (SEQ ID NO:37); Schatz, supra); mitochondrial intermembrane space sequences (yeast cytochrome c1; MFSMLSKRWAQRTLSKSFYSTATGAASKSGKLTQKLVTAGVAAAGITASTLLYADSLTAE AMTA (SEQ ID NO:38); Schatz, supra) or mitochondrial outer membrane sequences (yeast 70 kD outer membrane protein; MKSFITRNKTAILATVAATGTAIGAYYYYNQLQQQQQRGKK (SEQ ID NO:39); Schatz, supra).–

Paragraph beginning at page18, line 31, has been amended as follows:

– The target sequences may also be endoplasmic reticulum sequences, including the sequences from calreticulin (KDEL (SEQ ID NO:40); Pelham, Royal Society London Transactions B; 1-10 (1992)) or adenovirus E3/19K protein (LYLSRRSFIDEKKMP (SEQ ID NO:41); Jackson et al., EMBO J. 9:3153 (1990). –

Paragraph beginning at page 19, line 1, has been amended as follows:

– Furthermore, targeting sequences also include peroxisome sequences (for example, the peroxisome matrix sequence from Luciferase; SKL; Keller et al., PNAS USA 4:3264 (1987)); farnesylation sequences (for example, P21 H-ras 1; LNPPDESGPGCMSCK**C**VLS (SEQ ID NO:42), with the bold cysteine farnesylated; Capon, supra); geranylgeranylation sequences (for example, protein rab-5A; LTEPTQPTRNQ**CC**SN (SEQ ID NO:43), with the bold cysteines geranylgeranylated; Farnsworth, PNAS USA 91:11963 (1994)); or destruction sequences (cyclin B1; RTALGDIGN (SEQ ID NO:44); Klotzbucher et al., EMBO J. 1:3053 (1996)). –

Paragraph beginning at page 19, line 25, has been amended as follows:

– Suitable secretory sequences are known, including signals from IL-2 (MYRMQLLSCIALSLALVTNS (SEQ ID NO:45); Villinger et al., J. Immunol. 155:3946 (1995)), growth hormone (MATGSRTSLLLAFGLLCLPWLQEGSAFPT (SEQ ID NO:46); Roskam et al., Nucleic Acids Res. 7:30 (1979)); preproinsulin (MALWMRLLPLLALLALWGPDPAAAFVN (SEQ ID NO:47); Bell et al., Nature 284:26 (1980)); and influenza HA protein (MKAKLLVLLYAFVAGDQI (SEQ ID NO:48); Sekiwawa et al., PNAS 80:3563)), with cleavage between the non-underlined-underlined junction. A particularly preferred secretory signal sequence is the signal leader sequence from the

secreted cytokine IL-4, which comprises the first 24 amino acids of IL-4 as follows: MGLTSQLLPPLFFLLACAGNFVHG (SEQ ID NO:49).–

Paragraph beginning at page19, line 33, has been amended as follows:

– In a preferred embodiment, the fusion partner is a rescue sequence. A rescue sequence is a sequence which may be used to purify or isolate either the peptide or the nucleic acid encoding it. Thus, for example, peptide rescue sequences include purification sequences such as the His_6 (SEQ ID NO:50) tag for use with Ni affinity columns and epitope tags for detection, immunoprecipitation or FACS (fluoroscence-activated cell sorting). Suitable epitope tags include myc (for use with the commercially available 9E10 antibody), the BSP biotinylation target sequence of the bacterial enzyme BirA, flu tags, lacZ, GST, and Strep tag I and II. –

Paragraph beginning at page 20, line 9, has been amended as follows:

– In a preferred embodiment, the fusion partner is a stability sequence to confer stability to the peptide or the nucleic acid encoding it. Thus, for example, peptides may be stabilized by the incorporation of glycines after the initiation methionine (MG or MGG0), for protection of the peptide to ubiquitination as per Varshavsky's N-End Rule, thus conferring long half-life in the cytoplasm. Similarly, two prolines at the C-terminus impart peptides that are largely resistant to carboxypeptidase action. The presence of two glycines prior to the prolines impart both flexibility and prevent structure initiating events in the di-proline to be propagated into the peptide structure. Thus, preferred stability sequences are as follows: $MG(X)_nGGPP$ (SEQ ID NO:51), where X is any amino acid and n is an integer of at least four. –

Paragraph beginning at page 20, line 25, has been amended as follows:

– In a preferred embodiment, the fusion partner includes a linker or tethering sequence. Linker sequences between various targeting sequences (for example, membrane targeting sequences) and the other components of the constructs (such as the randomized peptides) may be desirable to allow the peptides to interact with potential targets unhindered. For example, useful linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:52) and $(GGGS)_n$, (SEQ ID NO:53) where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers such as the tether for the shaker potassium channel, and a large variety of other flexible linkers, as will be appreciated by those in the art. Glycine and glycine-serine polymers are preferred since both of these amino acids are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine polymers are the most preferred as glycine accesses significantly more phi-psi space than even alanine, and is much less restricted tan residues with longer side chains (see Scheraga, Rev. Computational Chem. III73-142 (1992)). Secondly, serine is hydrophilic and therefore able to solubilize what could be a globular glycine chain. Third, similar chains have been shown to be effective in joining subunits of recombinant proteins such as single chain antibodies. –

Paragraph beginning at page 22, line 9, has been amended as follows:

– The present invention has specifically contemplated each and every possible variation of polynucleotide that could be made by selecting combinations based on the possible codon choices, and all such variations are to be considered specifically disclosed and

equivalent to the sequences of Figures 2 and 3 (SEQ ID NOS:1, 3-4). Codons are preferably selected to fit the host cell in which the enzyme is being produced; that is, codon usage for yeast is used to express in yeast; codon usage for mammalian cells is used to express in mammalian cells; etc. Selection of codons to maximize expression of proteins in a heterologous host is a known technique. –

Paragraph beginning at page 54, line 3, has been amended as follows:

– A number of cyclin destruction boxes are known in the art, for example, cyclin A has a destruction box comprising the sequence RTVLGVIGD (SEQ ID NO:54); the destruction box of cyclin B1 comprises the sequence RTALGDIGN (SEQ ID NO:55). See Glotzer et al., Nature 349:132-138 (1991). Other destruction boxes are known as well: YMTVSIIDRFMQDSCVPKKMLQLVGVT (SEQ ID NO:56; rat cyclin B); KFRLLQETMYMTVSIIDRFMQNSCVPKK (SEQ ID NO:57; mouse cyclin B); RAILIDWLIQVQMKFRLLQETMYMTVS (SEQ ID NO:58; mouse cyclin B1); DRFLQAQLVCRKKLQVVGITALLLASK (SEQ ID NO:59; mouse cyclin B2); and MSVLRGKLQLVGTAAMLL (SEQ ID NO:60; mouse cyclin A2). –

Paragraph beginning at page 69, line 25, has been amended as follows:

– Retroviral constructs were based on a pCGFP vector that carries a composite CMV promoter fused to the transcriptional start site fo the MMLV R-U5 region of the LTR, and extended packaging sequence, deletion of the MMLV gag start ATG, and a multiple cloning region encoding human codon-optimized EGFP (Clontech, Palo Alto, CA) and a Kozak consensus start, described in Kozak *Cell* 44:283-292. The vector used to express flag tagged EGFP, pEf, is identical to pCGFP but has additional restriction sites in the

open reading frame of EGFP (resulting in 8 non-human optimized codons) and a Flag tag fused to the C-terminus of EGFP with the linker EEAAKA (SEQ ID NO:61). –

Paragraph beginning at page 70, line 3, has been amended as follows:

pR and pP are retroviral expression vectors containing human codon-optimized *Renilla muelleri* and *Ptilosarcus gurneyi* GFPs (containing 9 and 11 non-optimized codons, respectively, to introduce restriction sites). Each has a Kozak consensus start and backbone vector sequence identical to that of pCGFP and pEf. These vectors were made by annealing and ligating 20 synthetic oligonucleotides (10 forward, 10 reverse for each GFP gene) creating a dsDNA fragment for each sequence shown in Table 1. These fragments were PCR amplified with respective primers:

R forward, 5' -

GATCATAGAATTCGCCACCATGGGCAGCAAGCAGATCCTGAAGAACACCTGCCTG

(SEQ ID NO:62);

P forward, 5'-

GATCATAGAATTCGCCACCATGGGCAACCGCAACGTGCTGAAGAACACCGGCCTG

(SEQ ID NO:63);

R and P reverse, 5'-

ATGATCGCGGCCGCTACACCCACTCGTGCAGGGATCCCAGGGGCTTGCCGATG

(SEQ ID NO:64);

and cloned into the EcoRI/NotI restriction sites of pEf (replacing the Ef coding region). C-terminal Flag tags were added to these GFPs through BamHI/NotI sites using annealed primers with sticky overhangs:

Forward, 5' -

GATCCCTGCACGAGTGGGTGGAGGAGGCCGCCAAGGCCGACTACAAGGACGACGA
CGACAAGTAGGCCCGTGAGGCCCTAAGC (SEQ ID NO:65);

Reverse, 5' -

GGCCGCTTAGGGCCTCACGGGCCTACTTGTCGTCGTCCTTGTAGTCGGCCTTGGCG
GCCTCCTCCACCCACTCGTGCAGG (SEQ ID NO:66);

creating Rf and Pf. pRcDNA was made by removing the *R. muelleri* cDNA gene from pET-34 Native *Renilla muelleri* GFP (Prolume Ltd., Pittsburg, PA) by PCR amplification with primers:

Forward, 5' -

GATCATGAATTCGCCATGAGTAAACAAATATTGAAGAACACT (SEQ ID NO:67);

Reverse, 5' -

TAGATCGCGGCCGCTTAAACCCATTCGTGTAAGGATCCTAGTGG (SEQ ID NO:68);

and cloning into the EcoRI/NotI sites of pEf. Vectors containing codon optimized *R. muelleri* GFP with a linker-HA tag-linker sequence inserted into each position A-F were created by the PCR sew technique of two fragments using primers shown above (R

forward and R reverse). The two fragments for A-F were made by PCR amplification of the 5' section of R with respective primers:

R forward, shown above;

A reverse, 5' -

CTGGCGTAGTCGGGCACGTCGTAGGGGTAGCCACCGCCCTGGCCCTCGTAGCGCA GGGTGCGCTCGTAC (SEQ ID NO:69);

B reverse, 5' -

CTGGCGTAGTCGGGCACGTCGTAGGGGTAGCCACCGCCCTGGCCCTCGATCAGGT TGATGTCGCTGCGG (SEQ ID NO:70);

C reverse, 5' -

CTGGCGTAGTCGGGCACGTCGTAGGGGTAGCCACCGCCCTGGCCGTTCATGTACA TGGCCTCGAAGCTG (SEQ ID NO:71);

D reverse, 5' -

CTGGCGTAGTCGGGCACGTCGTAGGGGTAGCCACCGCCCTGGCCGTTAAGCTTGT ACACAGGATCACC (SEQ ID NO:72);

E reverse, 5' -

CTGGCGTAGTCGGGACGTCGTAGGGGTAGCCACCGAAATGGAAGAAATTGCTCTTC ATCAGGGTCTTC (SEQ ID NO:73);

F reverse, 5' -

CTGGCGTAGTCGGGCACGTCGTAGGGGTAGCCACCGCCCTGGCCGCCGCCGTCCT

CCACGTAGGTCTTC (SEQ ID NO:74);

and the 3' section of R with respective primers:

A forward, 5' -

CCTACGACGTGCCCGACTACGCCAGCCTGGGCCAGCAGGTGGAGGCGACGGCGG

CCTGGTGGAGATCCGCA (SEQ ID NO:75);

B forward, 5' -

CCTACGACGTGCCCGACTAGCCAGCCTGGGCCAAGCAGGTGGAGGCGACAAGTTC

GTGTACCGCGTGGAGT (SEQ ID NO:76);

C forward, 5' -

CCTACGACGTGCCCGACTACGCCAGCCTGGGCCAAGCAGGTGGAGGCAACGGCGT

GCTGGTGGGCGAGGTGA (SEQ ID NO:77);

D forward, 5' -

CCTACGACGTGCCCGACTACGCCAGCCTGGGCCAAGCAGGTGGAGGCAGCGGCAA

GTACTACAGCTGCCACA (SEQ ID NO:78);

E forward, 5' -

CCTACGACGTGCCCGACTACGCCAGCCTGGGCCAAGCAGGTGGAGGCGTGGTGAA

GGAGTTCCCCAGCTACC (SEQ ID NO:79);

F forward, 5' -

CCTACGACGTGCCCGACTACGCCAGCCTGGGCCAAGCAGGTGGAGGCTTCGTGGA GCAGCACGAGACCGCCA (SEQ ID NO:80). The PCR sewed fragments were put into the EcoRI/NotI sites of pEf. –

Paragraph beginning at page 71, line 36, has been amended as follows:

– The bacterial expression vector for purification of *Ptilosarcus* GFP was created by PCR amplfication of pP with primers:

forward, 5' -

AGATCATAGATCTATGGGCAACCGCAACGTGCTGAAGAACACCGGCCTG (SEQ ID NO:81);

P reverse, shown above.

Digestion of the fragment withBglII/NotI and ligation into the BamHI/NotI restriction sites of pGEX6P-1 (Pharmacia Biotech, Piscataway, New Jersey). The vector containing *R. muelleri* GFP with C10G and C35E mutations (observed to aid in the folding of the protein in bacteria) was created by PCR sewing together a fragment created by annealing and extending primers:

forward, 5' -

AGATCATAGATCTGAATTCATGGGCAGCAAGCAGATCCTGAAGAACACCGGCCTGC AGGAGGTGATGAGCTACAAGGTGACCTGGAGG (SEQ ID NO:82);

reverse, 5' -

GCCAACAGGATGTTGCCCTTGCCCTCGCCCTCCATGGTGAACACGTGGTTGTTAAC GATGCCCTCCAGGTTCACCTTGTAGCTCATCAC (SEQ ID NO:83);

R reverse, shown above.

The sewed product was digested BglII/NotI and ligated into the BamHI/NotI sites of pGEX6P-1. –

Paragraph beginning at page 73, line 19, has been amended as follows:

– All components used for purification of the GFP gene products were from Pharmacia Biotec (Piscataway, NJ) except as noted. The human codon-optimized gene for each protein was expressed in BL21 TIL codon plus (DE3) *E. coli* (Stratagene, San Diego, CA) as a fusion protein with glutathione S-transferase from pGEX6p-1 derived vectors. Each protein was purified using Glutathione Sepharose 4B beads as per the manufacturer's directions, and the mature GFP was removed from the protein with Precision Protease. The purified proteins ran as single bands by SDS-PAGE and appeared as single peaks of the expected molecular mass by MALDI-TOF mass spectometry on a Bruker Reflex III instrument (Bruker Daltonics, Billerica, MA). Due to the cloning strategy, purified *R. muelleri* GFP has the amino acids PLGSEF- (SEQ ID NO:84) and *Ptilosarcus* GFP the residues GPLGS- (SEQ ID NO:85) fused to their N-termini. Purified recombinant EGFP was from Clontech (Palo Alto, CA). –